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ENU Mutagenic Screen for Susceptibility and Resistance to *Streptococcus Pneumoniae*

Start Date: 8/01/02 End Date: 07/03
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PROJECT GOALS

- The main goal of the DARPA HOT program is to determine the ability of the host organism to provide protection against bacterial and viral infection. The genetic pathways that control susceptibility and resistance to bacterial infection have remained poorly understood, because of the lack of expertise in the development of techniques capable of identifying factors that are involved in the process. The proposal outlined here will use chemical mutagenesis with *N*-ethyl-*N*-nitrosourea (ENU) combined with the resources of mouse genomics to identify genes that are involved in the susceptibility and resistance to bacterial infection. Male Balb/c mice (G0) will be subjected to ENU mutagenesis using standard protocols and crossed to female C57BL/6 mice to generate G1 offspring. The G1 generation will be subjected to a lethal dose (LD_{100}) of *streptococcus pneumonia* and animals that are resistant and highly susceptible will be identified. The G0 males of the resistant G1 offspring will be outcrossed onto the C57BL/6 background to facilitate the genetic mapping of the mutations by haplotype analysis. The animals will be analyzed to identify the chromosomal locus and eventual identification of the gene(s) responsible for susceptibility and resistance to bacterial infection.

TECHNICAL APPROACH

- In order to identify genes that will confer resistance to streptococcus pneumoniae, mice will be subjected to *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis followed by exposure to a lethal dose of *streptococcus pneumoniae*. Male Balb/c mice will be purchased from Jackson Laboratories and allowed to acclimate for 1 week. ENU, obtained from Sigma Chemical Co., (N-3385) in Isopac vials containing approximately 1.0 g of ENU will be dissolved in 10 ml of 95% ethanol for 1 hour. Ninety mls of phosphate-citrate buffer (0.1 M Na_2HPO_4/0.05 M citrate, pH 5.0) will be added and the resulting 10 mg/ml solution of ENU will be injected into male Balb/c mice. Approximately 100 male Balb/c mice (9-10 weeks old) will be injected with ENU in three doses of 100 mg/kg body weight administered once a week for three weeks. Animals are initially infertile and will regain fertility after 7-10 weeks (15). To screen for dominant mutations that would affect resistance to *streptococcus pneumoniae*, mutagenized males will be crossed to Balb/c females to generate G1 males. The G1 progeny will be screened for susceptibility to streptococcus pneumoniae. This will be accomplished by challenging the mice with an i.p. injection of 10^6 CFU of *streptococcus pneumoniae* (D39 or M24)(16,17). Mice will

be observed every 4 hours for the first 96 hours and then twice daily for the next 96 and then daily until 21 days. Under the conditions listed 100% of the wildtype mice will die within 96 hours. Viable animals will be subjected to a second round of challenge to verify the resistance to *S. pneumoniae*. G0 males from the resistant offspring will be identified and bred for mapping studies.

- Mutations will be mapped by using DNA from the tail snip from 20-50 adult mice presumed to be heterozygous for the mutation because they were parents of the mutant mice (15). The markers listed below will be used in an initial genome scan at 40-centimorgan (cM) intervals, if no clear linkage is observed, additional markers at 20-cM intervals will be tested. If linkage is observed, it will be confirmed with additional carrier animals and mutant offspring.

- Additionally, Dr. Rick Lyons in the Department of Medicine at the University of New Mexico, will test the effects of *bacillus anthracis*, *tularemia*, small pox virus and influenza on the individual lines that show resistance or susceptibility to *streptococcus pneumoniae*.

MAJOR CHALLENGES

- Identify mutants that are resistant to bacterial infection.
- Identify mutants that are susceptible to bacterial infection.
- Rapidly identify region of interest for the genes that confer resistance and susceptibility to bacterial infection.

MILESTONES/TASKS

- Mice were challenged with a systemic infection of *S. pneumoniae* strain D39. Balb/c mice were challenged with a dose of 10^6 colony-forming units (CFUs) via i.p. Survival curve for Balb/c mice treated with S. pneumoniae are shown in Figure 1.

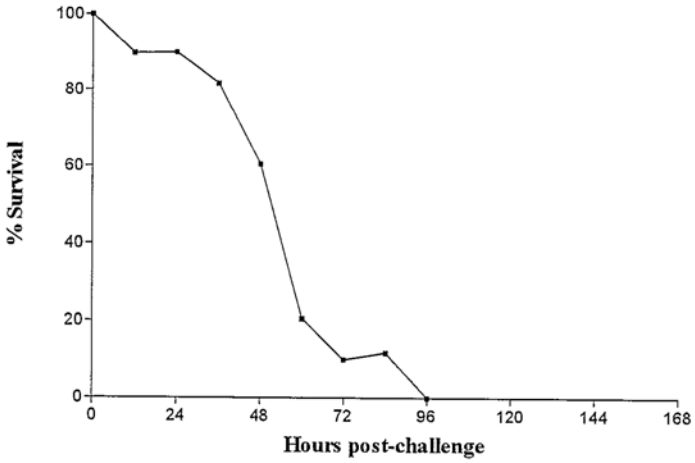

Figure 1. Survival curve for *S. pneumoniae* treated Balb/c mice after IP challenge with 10^6 CFUs of *S. pneumoniae*. Each point is the mean of 6 animals treated with *S. pneumoniae*.

Male Balb/c mice were injected (50 mice) with ENU (100 mg/kg per week for 3 weeks) in August 2002. The mice were maintained for 12 weeks without any further treatment to allow for the mice to transition through a sterility phase into the fertility phase to allow for reproduction. At the beginning of December 2002, the mice were bred onto a C57BL/6 strain to generate the G1 offspring to screen for susceptibility or resistance to *S. pneumoniae*. By the end of December 2002, 237 mice were born to 29 mice and 21 more were in various stages of pregnancy. The mice will be allowed to mature to 8 weeks of age and then will be tested.

FUTURE PLANS

- Once we have identified a breeder that has generated mice that demonstrate increased susceptibility or resistance to *S. pneumoniae* these mice will be bred to generate a line of mice to search for the gene of interest.
- In a second round of ENU mutagenesis, the G1 offspring will undergo a second round of breeding in order to maintain the G1 colony for easier selection of the mutants.
- Begin screening mutant strains of mice that we believe may be important in the process of increased resistance or susceptibility to *S. pneumoniae*. Obtain these strains of mice and begin breeding and testing of strains.

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